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Persistence of allospecific helper T cells is required for maintaining autoantibody formation in lupus-like graft-*versus*-host disease

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SUMMARY

Induction of a graft-*versus*-host (GVH) reaction (GVHR) in non-irradiated (C57BL/10ScSn × DBA/2)F1 mice (BDF1) with DBA/2 lymphoid cells leads to chronic GVH disease (GVHD). One of the pathological alterations of this type of GVHD is hyperplasia of host B cells with production of lupus-like autoantibodies. This hyperstimulation of host B cells has previously been demonstrated to be induced by alloreactive donor T helper cells that were also proposed to maintain it. We provide three pieces of experimental evidence in support of this concept. First, treatment of mice with chronic GVHD by injection of monoclonal anti-Thy-1.2 antibodies, performed at week 6 after the injection of C57BL/6 lymphoid cells into (C57BL/6 × C57BL.bm12)F1 mice led to a significant decrease in the titre of anti-nuclear antibodies. Second, $CD4^+$ donor T cells persisted in BDF1 mice with GVHD (GVHF1) for at least 10 weeks after the induction of GVHR; these T cells showed alloreactive helper activity against $H\text{-}2^b$ MHC determinants of the opposite parent *in vitro*. Third, T cells of GVHF1 mice, obtained 2 months after the induction of GVHR and transferred into normal secondary recipients, induced signs of chronic GVHD in DBF1 but not in DBA/2 mice. The combined results show that persisting donor T helper cells in GVHF1 mice retain their alloreactivity towards H-2 class II antigens for a long time after the induction of GVHR and they strongly suggest that these T cells are also the driving force behind the production of lupus-like autoantibodies at the late stage of chronic GVHD.

Keywords graft-*versus*-host disease autoimmunity allospecific helper T cells

INTRODUCTION

Induction of a graft-*versus*-host (GVH) reaction (GVHR) in non-irradiated F1 mice by i.v. injection of parental strain lymphoid cells may lead to chronic GVH disease (GVHD) (Gleichmann *et al.*, 1984). Chronic GVHD is characterized by formation of autoantibodies, immune complex glomerulonephritis and histopathologic lesions that resemble vascular collagen diseases. The autoantibodies are not organ-specific and are mainly of the IgG isotype. They react with erythrocytes, thymocytes and various nuclear antigens including dsDNA (van Rappard-van der Veen *et al.*, 1983; Rolink, Pals & Gleichmann, 1983a, 1983b; Pals, Radaszkiewicz & Gleichmann, 1984b; Pals *et al.*, 1985; Kimura, van Rappard-van der Veen & Gleichmann, 1986). In contrast, autoantibodies reacting to self-antigens involved in organ-specific autoimmunity, such as antibodies to thyroglobulin (van Rappard-van der Veen *et al.*, 1984; Kuppers *et al.*, 1988), do not normally develop in chronic GVHD. Thus, the autoantibodies in chronic GVHD carry the features of autoantibodies characteristic of systemic lupus erythematosus (SLE).

Chronic or lupus-like GVHD is a model of murine SLE induced in genetically normal mice. Requirements for the inductive phase of lupus-like GVHD have been extensively studied. Two absolute requirements are the presence in the donor cell inoculum of alloreactive T helper cells and in the F1 hybrid recipient of an intact B cell system (Van Rappard-Van Der Veen *et al.*, 1983). An MHC class II disparity is an additional requirement in non-irradiated F1 recipients (Kimura *et al.*, 1986). The fact that the donor T helper cells, needed for induction of this syndrome react against the allogeneic MHC class II antigens of the F1 recipient but lack obvious specificity for autoantigens, suggests that in SLE-like autoantibody formation a bystander type of T–B cell cooperation is involved. According to this concept, formation of SLE-like autoanti-

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bodies is T helper cell-dependent, but the T helper cells do not recognize the self-antigens, such as nuclear antigens, to which autoantibodies are formed. Thus, the autoantibody formation in systemic autoimmunity seems to differ from that in organ-specific autoimmunity where 'linked' T–B collaboration appears to be involved (Gleichmann *et al.*, 1984).

Analysis of the development of lupus-like GVHD has been restricted to the inductive phase of GVHR. The extent to which parental strain allospecific T helper cells are also needed for maintaining the autoantibody formation throughout the course of lupus-like GVHD has not been tested. We now provide evidence that allospecific donor T helper cells persist for at least 3 months after the induction of GVHR in the F1 recipient and that they maintain the formation of anti-nuclear antibodies (ANA).

MATERIALS AND METHODS

Mice

DBA/2 (H-2^d, Ly-1.1, Ly-2.1) and (C57BL/10ScSn × DBA/2) F1 (BDF1) hybrid mice were purchased from Olac (Bicester, UK). C57BL/6Kh (B6, H-2^b, Ly-1.2, Ly-2.2) and B6.C-H-2^{bm12} (bm12) were maintained in our animals facilities. The bm12 strain differs from the original B6 strain by a mutation at the H-2 I-A locus (McKenzie *et al.*, 1979). (B6 × bm12)F1 mice were bred in our animals facilities. Female mice aged 6–10 weeks were used.

Induction of lupus-like GVHD

GVHR was induced by two i.v. injections of lymphoid cells from DBA/2 donors into non-irradiated F1 recipients. The two injections were separated by a 1-week interval. Each inoculum contained 50×10^6 live spleen (2/3) and lymph node (1/3) cells.

Detection of anti-nuclear antibodies

At intervals of 3 weeks, sera of GVHF1 mice were tested for the presence of IgG ANA by indirect immunofluorescence as described (Kimura *et al.*, 1986). Briefly, murine liver sections were incubated with diluted sera. The liver sections were washed and incubated with fluorescein-conjugated rabbit anti-mouse IgG serum (Central Laboratory of the Blood Transfusion Service (CLB), Amsterdam, The Netherlands). The ANA titre was used as an indicator of the severity of lupus-like GVHD (Kimura *et al.*, 1986).

Preparation of nylon/wool non-adherent spleen cells

Spleen cells were incubated on a nylon/wool column at 37°C for 30 min and the non-adherent fraction was collected; 80–95% of the nylon/wool non-adherent cells were Thy-1.2$^+$.

Antibodies

Polyclonal murine anti-H-2^b and anti-H-2^d alloantibodies were prepared by injection of B10 (H-2^b) lymphoid cells into B10.D2 (H-2^d) and of B10.D2 cells into B10 mice respectively. The sera obtained were specific both in the immunofluorescence assay and in the cytotoxicity assay (data not shown). Anti-Lyt-1.1 (catalog no. CL 8911) and anti-Lyt-2.1 (anti-CD8, catalog no. CL 8921), both murine monoclonal IgG2a antibodies, were obtained from Cedarlane (Hornby, Canada). Anti-L3T4 (anti-CD4), a murine monoclonal IgM antibody, was kindly given by Dr H. R. MacDonald, Lausanne, Switzerland. Anti-Thy-1.2 clone F7D5 (Serotec, Oxford, UK) murine monoclonal IgM antibody, was used in all *in vitro* experiments. Anti-Thy-1.2 murine monoclonal IgG2a antibody (Opitz *et al.*, 1982), kindly given by Dr H. G. Opitz (Bayer, FRG) was used for anti-Thy-1 treatment in vivo.

Depletion of T cells in vivo

On days 35, 37 and 39 after the injection of B6 lymphoid cells into (B6 × bm12)F1 recipients, GVHF1 mice were injected intravenously with 0·2 ml of ascitic fluid containing 150–250 μg of monoclonal anti-Thy-1.2 IgG2a antibody.

Irradiation of GVHF1 spleen cells

GVHF1 spleen cells were exposed to 20 Gy of 662 KeV gamma rays from a ^{137}Cs source at a dose of 3·6 Gy/min.

Immunofluorescence analysis of cell suspensions

Suspensions of 500×10^3 cells were incubated with appropriately diluted antibodies in a total volume of 50 μl. Cells were washed three times and incubated with fluorescein-labelled goat anti-mouse IgG antibodies (CLB GM 17-01-FO3, CLB). Immunofluorescence was measured on a flow cytometer (Ortho FC200-4800A, Ortho Instruments, Westwood, MA).

Treatment of spleen cells with antibody and complement

Cell suspensions of 60×10^6 viable cells/ml were incubated for 45 min on ice with 1/200-diluted anti-Thy-1.2 or anti-CD4, or 1/10-diluted anti-CD8, or anti-H-2^b. After the incubation period guinea pig complement (ORAY 20/21, Behringwerke, Marburg, FRG) was added to 30×10^6 cells/ml at a final dilution of 1/10. The cells were further incubated for 45 min at 37°C. Cells were washed twice, and the number of viable cells was determined by trypan blue exclusion.

Primary antibody response in vitro *to sheep erythrocytes*

Primary B cell responses *in vitro* to sheep erythrocytes were assessed by plaque-forming cell (PFC) response as described (Pals *et al.*, 1984a). Briefly, $0{\cdot}5 \times 10^6$ sheep erythrocytes and 3×10^6 spleen cells or normal BDF1 mice that had been treated *in vitro* with anti-Thy-1.2 and complement (B cells) were cultured with graded numbers of T cells of DBA/2, normal BDF1, or GVHF1 mice. Cultures were performed in 24-well tissue culture plates in 0·3 ml Iscove's modified Dulbecco's medium supplemented with penicillin, streptomycin, 2-mercaptoethanol and 10% fetal calf serum (FCS). The direct anti-sheep erythrocytes PFC response was performed on day 4 of culture by the Jerne plaque assay.

Statistical analysis

The Mann–Whitney *U*-test, a non-parametric test for independent samples, was used for analysis of differences between groups of mice that were treated with anti-Thy-1.2 *in vivo* and their control counterparts. The Wilcoxon matched-pairs signed ranks test, a non-parametric test for related samples, was used for analysis of differences within groups before and after treatment with anti-Thy-1.2, or with control ascitic fluid.

RESULTS

Decline of ANA titres after treatment of GVHF1 mice with anti-Thy-1.2 in vivo

GVHR was induced by the injection of B6 lymphoid cells into (B6 × bm12)F1 mice. On days 35, 37 and 39 after the induction of GVHR, GVHF1 mice were injected intravenously with 200 μl ascitic fluid containing 150–250 μg of monoclonal anti-Thy-1.2 IgG2a antibody, or with control ascitic fluid. Data of this experiment are shown in Fig. 1. The ANA titres in GVHF1 mice treated with anti-Thy-1.2 were significantly lower at 9 and 13 weeks after the induction of GVHR than those in control mice ($P < 0.001$, Mann–Whitney U-test). The ANA titres in the anti-Thy-1.2-treated mice at 9 and 13 weeks were also significantly lower than those at 4 weeks after the induction of GVHR ($P < 0.01$, Wilcoxon's two-tailed matched-pairs signed ranks test). In the control mice, ANA titres did not differ significantly at these three time-points and were indistinguishable from those of the anti-Thy-1.2-treated mice at 4 weeks after the induction of GVHR, i.e. preceding treatment.

Cell-surface markers of donor cells in mice with chronic GVHD

Immunofluorescence assays showed that at 10 weeks after the induction of GVHR in BDF1 mice GVHF1 spleens contained 4–9% of surface-immunoglobulin-negative, H-2^{b-}, H-2^{d+} cells (data not shown). Such a cell population could not be found in normal BDF1 mice. This cell population is clearly derived from the injected DBA/2 cells used to induce GVHR. In order to analyse cell-surface markers of these donor cells, GVHF1 spleen cells were passed through a nylon/wool column and the non-adherent cells were treated with anti-H-2^b and complement. FACS analysis of these nylon/wool non-adherent H-2^{b-} cells with a panel of monoclonal antibodies (anti-Thy-1.2, anti-Ly-1.1, anti-CD4 (L3T4) and anti-CD8 (Ly-2.1)) showed that most of these cells were $CD4^+$ and $CD8^-$ (Table 1). According to the results of these experiments 4–9% of GVHF1 spleen cells were $CD4^+$, $CD8^-$ T cells of donor origin at 10 weeks after the induction of GVHR.

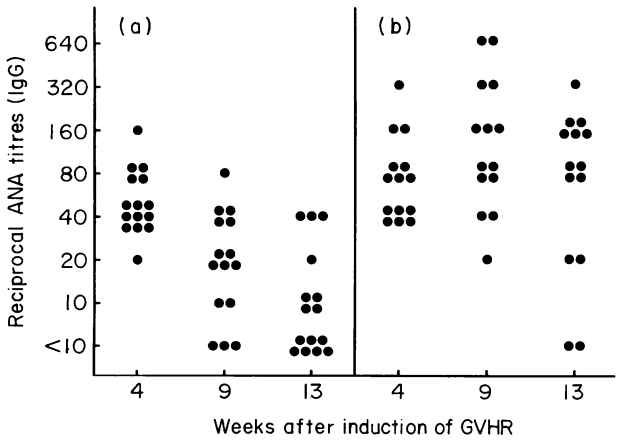

Fig. 1. Production of IgG anti-nuclear antibodies (ANA) in GVHF1 mice, as demonstrable in serum, is T cell dependent. (B6 × bm12)F1 mice were injected with B6 lymphoid cells. On 35, 37 and 39 days after the induction of GVHR mice received i.v. injections of either (a) monoclonal anti-Thy-1.2 ($n = 15$); or (b) control ascitic fluid ($n = 14$).

Table 1. Cell surface markers of H-2^b-negative spleen cells of GVHF1 mice

Antibody	positive cells (%)
Anti-H-2^d	99
Anti-Thy-1·2	64–89
Anti-Lyt-1·1*	96–97
Anti-CD4	71–90
Anti-CD8	1
NMS	0

Nylon/wool non-adherent spleen cells of GVHF1 mice, obtained at 10 weeks after the injection of DBA/2 lymphoid cells into BDF1 mice, were first treated with anti-H-2^b and complement. Next, cell surface markers of the remaining cell population were analysed with the monoclonal antibodies indicated.

Data obtained with seven individual mice are summarized. Results were corrected for 20% positive cells due to surface immunoglobulin-positive cells and/or H-2^b-positive cells with residual anti-H-2^b antibodies left that survived treatment and/or dead cells.

*Anti-Lyt-1·1 is used here as a T cell marker for this surface immunoglobulin-negative cells population since DBA/2 T cells have been reported to express only low amounts of the Thy-1·2 antigen (Del Guercio *et al.*, 1982).

NMS, normal mouse serum.

Demonstration of allohelper activity of donor T cells that persist during chronic GVHD

GVHF1 spleen cells obtained at 10 weeks after the induction of GVHR in BDF1 mice did not affect the anti-sheep erythrocytes response of normal BDF1 cells, i.e. the response of BDF1 cells was neither enhanced nor suppressed (data not shown). However, nylon/wool non-adherent GVHF1 spleen cells, treated with anti-H-2^b and complement enhanced the primary antibody response of normal BDF1 B cells to sheep erythrocytes. Data of a representative experiment are shown in Fig. 2. Irradiation of the GVHF1 cells prevented helper activity. The helper activity of the nylon/wool non-adherent H-2^b-negative GVHF1 spleen cells was specifically directed against H-2^b MHC determinants on target cells, because it was not provided to DBA/2 B cells (data not shown).

Further analysis of the allospecific donor T helper cells

Nylon/wool non-adherent H-2^b-negative spleen cells of GVHF1 mice, which provided allo-helper activity to normal BDF1 B cells *in vitro*, were treated with either anti-Thy-1.2, anti-CD4 or anti-CD8 and complement. The remaining viable cells were tested for allospecific helper activity. Data of a representative experiment are shown in Fig. 3. Treatment of the nylon/wool non-adherent H-2^b-negative GVHF1 spleen cells with anti-Thy-1.2 or anti-CD4 and complement abolished their allospecific helper activity for normal BDF1 B cells, whereas treatment with anti-CD8 did not affect this activity. From this experiment we concluded that the H-2^b-negative, Thy-1.2^+, $CD4^+$ and $CD8^-$ donor-derived cells from mice with lupus-like GVHD were indeed responsible for the observed allospecific helper activity.

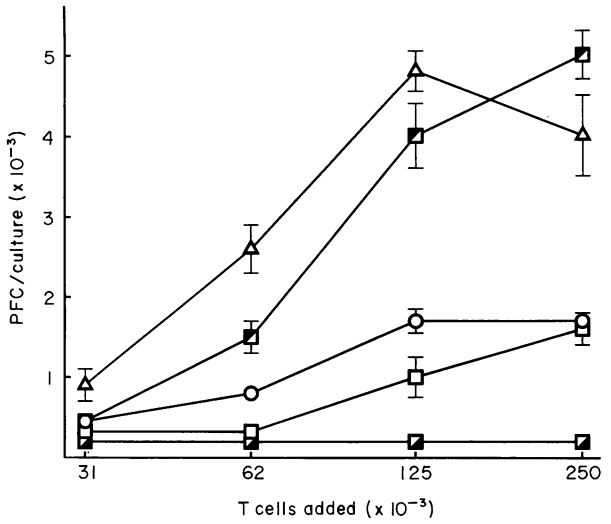

Fig. 2. Helper activity in GVHF1 T cells is enriched in the H-2^b-negative fraction. T cells of DBA/2 (△), normal BDF1 (○) or GVHF1 mice were added to cultures of normal BDF1 B cells and sheep erythrocytes. GVHF1 spleen cells were obtained 70 days after the induction of GVHR in BDF1 mice with DBA/2 lymphoid cells and had been treated with either normal mouse serum and complement (□), anti-H-2^b and complement (◩) or 20 Gy irradiation (◪). PFC, plaque-forming cells.

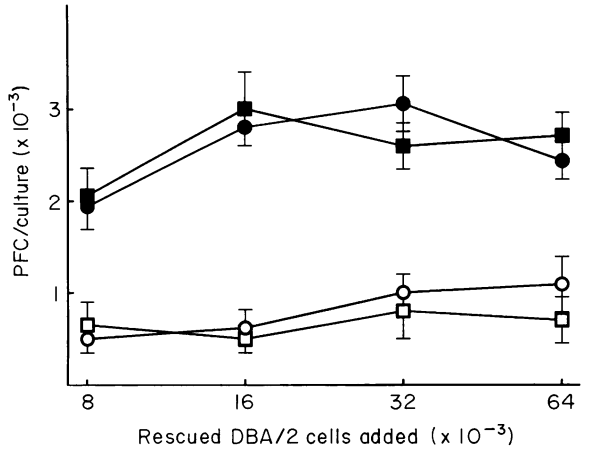

Fig. 3. The phenotype of alloreactive donor T cells rescued from GVHF1 mice is Thy-1.2$^+$, CD4$^+$ and CD8$^-$. GVHF1 T cells obtained 70 days after the induction of GVHR in BDF1 mice with DBA/2 lymphoid cells were treated with anti-H-2^b and complement. Next, they were treated with anti-Thy-1.2 (□), anti-CD4 (○), anti-CD8 (■), or normal mouse serum (●) and complement. The remaining cells were added to cultures of normal BDF1 B cells and sheep erythrocytes. PFC, plaque-forming cells.

Induction of chronic GVHD by adoptive transfer to secondary BDF1 recipients of GVHF1 spleen cells

Spleen cells of GVHF1 mice, obtained 7 or 9 weeks after the induction of GVHR, were adoptively transferred to secondary recipients. Pooled data of two experiments are shown in Fig. 4. Transfer into DBA/2 secondary recipients, i.e. mice that were syngeneic with the parental donor strain used for the induction of the primary GVHD, did not lead to signs of chronic GVHD,

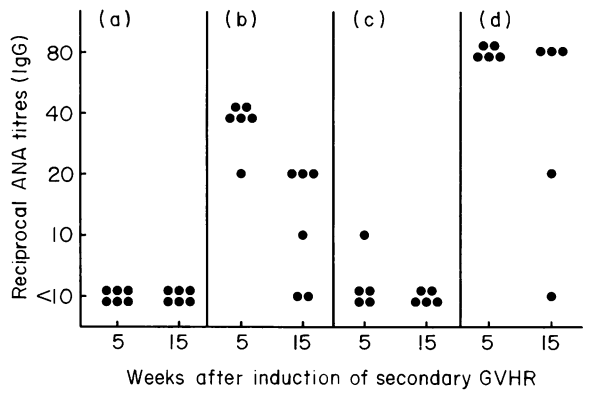

Fig. 4. Spleen cells from autoimmune GVHF1 mice adoptively transferred to secondary recipients induce *de novo* anti-nuclear antibodies (ANA) formation in BDF1, but not in DBA/2 secondary recipients. Primary GVHR was induced in BDF1 mice with DBA/2 lymphoid cells. At 8 weeks after induction of primary GVHR, 200 × 10^6 live spleen cells from primary GVHF1 mice were transferred to the secondary recipients. (a) untreated GVHF1 spleen cells transferred to DBA/2 mice; (b) untreated GVHF1 spleen cells transferred to BDF1 mice; (c) GVHF1 spleen cells were pretreated with anti-Thy-1·2 and complement; (d) GVHF1 spleen cells were pretreated with normal mouse serum and complement before being transferred to BDF1 mice.

whereas transfer into normal BDF1 mice did. The induction of chronic GVHD in the secondary recipients depended on the presence in the inoculum of T cells. Treatment of primary GVHF1 spleen cells with anti-Thy-1.2 and complement prior to transfer abolished their capacity to induce signs of chronic GVHD in secondary BDF1 mice.

DISCUSSION

Chronic or lupus-like GVHD, as monitored by high serum titres of IgG ANA, was induced in non-irradiated BDF1 mice by injection of lymphoid cells from DBA/2 donors. We found that the spleens of GVHF1 recipients contained 4–9% of CD4$^+$ CD8$^-$ donor T helper cells until at least 10 weeks after the induction of GVHR. Chimaerism by persistent donor lymphoid cells in F1 mice with lupus-like GVHD has been reported by Pals *et al.*, (1984b), Moser *et al.*, (1987) and Via, Sharrow & Shearer (1987). At 2 weeks after the induction of GVHR in Thy-1.2$^+$ (B6 × bm12)F1 mice, using spleen cells from Thy-1.1 congenic B6 donor mice, Moser *et al.*, (1987) found about 4% of the GVHF1 spleen cells to be CD4$^+$ T helper cells of donor origin. Via *et al.*, (1987) found about 3% of persisting CD4$^+$ and negligible amounts of CD8$^+$ donor T helper cells in non-irradiated BDF1 mice with GVHD induced by DBA/2 lymphoid cells.

The persistent donor T helper cells may be either the offspring of alloreactive T helper cells injected to induce GVHR, or they may have arisen in the recipient mice from donor stem cells present among the spleen cells used to induce GVHR. Notably, BDF1 mice injected with DBA/2 spleen and lymph node cells may become completely repopulated by donor lymphoid cells wtihout clinical signs of GVHD (van Rappard-Van der Veen *et al.*, 1983; Pals, Gleichmann & Gleichmann, 1984a). However, T helper cells derived from stem cells become tolerant for the MHC antigens of the host (Pals *et al.*, 1984a) and removal of stem cell-derived T cells by a short treatment with

anti-Thy-1 in vivo does not last more than 6 weeks (Opitz *et al.*, 1982). By contrast, such treatment of lupus-like GVHD was sufficient to reduce the ANA titre for at least 9 weeks. Thus, the available evidence strongly suggests that the T helper cells that maintain lupus-like GVHD are the offspring of the T helper cells used to induce GVHR.

Mice with chronic GVHD show high serum titres of antibodies characteristic of SLE. B cells that can produce such autoantibodies are part of the normal B cell repertoire. Naparstek *et al.*, (1986) detected autoantibodies characteristic of SLE even during the initial phase of an immune response to the antigen *p*-azophenyl arsonate. Although these autoantibodies were mainly of the IgM class and disappeared after the IgM to IgG switch of the response to the nominal antigen, the distinction between physiology and pathology need not always be clear-cut. In non-irradiated F1 mice with chronic GVHD the SLE-like autoantibodies demonstrated were IgG autoantibodies, indicating a sustained and T helper cell-dependent immune response. In murine models of genetically determined SLE, such as (NZB/W)F1 and BXSB mice, but not MRL/l mice, murine lupus is characterized by a hyper-responsiveness of B cells, which appears to be T helper cell dependent (reviewed by Theofilopoulos & Dixon, 1987). Murine lupus-like disease can also be induced in parental strain mice after neonatal induction of transplantation tolerance to semi-allogeneic F1 spleen cells (Goldman *et al.*, 1983). In this model both autoantibody-producing F1 donor B cells (Luzuy *et al.*, 1986) and allospecific host T helper cells (Goldman *et al.*, 1989) persist, and both cell populations are required for the development of autoimmunity and glomerulonephritis (Abramowicz *et al.*, 1988).

Our findings indicate that donor T helper cells in all likelihood induce and maintain the formation of SLE-like autoantibodies in chronic GVHD. First, we found that injection of anti-Thy-1.2 into GVHF1 mice with high serum titres of ANA led to a significant decrease in ANA titres. Second, donor T helper cells rescued from non-irradiated F1 mice with chronic GVHD showed allohelper activity to normal F1 B cells *in vitro*. Third, adoptive transfer of live T cells obtained from F1 mice with primary chronic GVHD to secondary F1 recipients, but not to DBA/2 mice, induced *de novo* formation of anti-nuclear autoantibodies. From these experiments we conclude that donor T helper cells retain their allospecific helper reactivity for at least 3 months after the initiation of GVHR. The latter two experiments, however, did not discriminate between donor and host T cells; therefore, no direct conclusion can be drawn as to whether it suffices to remove only donor T cells to diminish symptoms of lupus-like GVHD.

However, previous experiments (Gleichmann & Gleichmann, 1976; Gleichman, Gleichman & Wilke, 1976) have shown that T cells of the F1 host are not required for induction of lupus-like GVHD, but that their removal in fact greatly enhanced the development of this disease. Therefore it is very likely that the effects of T cells described here, i.e. induction of *de novo* autoantibody formation following adoptive transfer of GVHF1 T cells and decrease of ongoing autoantibody formation by treatment with anti-Thy-1.2, were due to T cells of the donor and not of the F1 host. Apart from allospecific T helper cells, other T cell populations may influence the production of autoantibodies. F1 mice with acute GVHD produced only small amounts of ANA by the time alloreactive cytotoxic T lymphocytes (CTL) could no longer be detected, although allospecific T helper cells were detectable throughout the observed period of 3 months (Pals *et al.*, 1984a, 1984b). Pals *et al.* (1984b) and Via & Shearer (1988) suggested that production of autoantibodies in acute GVHD is prevented by alloreactive CTL that kill many of the B cells of the host including the autoreactive ones.

We argue that the formation of SLE-like autoantibodies and hypergammaglobulinaemia are the result of a bystander type of cooperation between donor T helper cells and host B cells (Gleichmann *et al.*, 1984). In this type of T–B cooperation, although formation of SLE-like autoantibodies depends on T helper cells, the donor T helper cells do not recognize the self-antigens involved together with syngeneic H-2 molecules, but they recognize the allogeneic H-2 class II molecules on host cells. Then, of all B cells only the ones that have bound the appropriate antigen produce antibodies. This abnormal T–B cell cooperation does not seem to lead to increased relative frequencies of autoreactive B cells (Rolink, Radaszkiewicz & Melchers, 1987; Klinman Ishigabutso & Steinberg, 1988). Dziarski (1988) argues that a similar T–B cell mechanism, i.e. initial polyclonal activation of B cells followed by an expansion of the autoreactive clones caused by the availability of autoantigens, holds for murine models of genetically determined lupus-like disease. Autoantibodies that react to organ-specific antigens such as thyroglobulin may also be formed, provided that large amounts of the antigen are present at the time of GVHR induction (van Rappard-van der Veen *et al.*, 1984; Kuppers *et al.*, 1988).

Rolink, Gleichmann & Gleichmann (1983c) noted that eluates of kidneys from mice with lupus-like GVHD contain antibodies directed against antigenic structures of murine leukaemia virus (MuLV). MuLV and other endogenous or exogenous viruses might contribute to the stimulation of the host's immune system during ongoing GVHD and, hence, to the sings of GVHD. Irrespective of whether activated viruses contribute to the lesions of GVHD, one might argue that after the initial assault of alloreactive T cells the F1 recipient's immune system becomes so perturbed that tolerance to self-antigens is lost for a long time. If so, the mechanism of bystander T–B cooperation for SLE-like autoantibody formation would be much less important than assumed. We have now provided evidence, however, that donor T helper cells retain their allospecific reactivity during lupus-like GVHD. Therefore, bystander type of T–B cooperation appears to be an important factor for the ongoing production of SLE-like autoantibodies in chronic GVHD.

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